ACEPHATE

REVISED TOXICOLOGY CHAPTER FOR RED

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Human Health Assessment

1. Toxicology Assessment

The toxicity data base for Acephate is adequate and will support reregistration eligibility.

a. Acute Toxicity

The table below summarizes the acute toxicity studies on Acephate and the toxicity categories for the different routes of administration.

ACUTE TOXICITY DATA FOR ACEPHATE

Test	Results	Category
Acute Oral LD_{50} (Rat) MRID 00014675	945 mg/kg ♂ 866 mg/kg ♀	
Acute Oral LD_{50} Recalculation (Rat) MRID 00029686	1.4 g/kg ♂ 1.0 g/kg ♀	3
Acute Dermal LD_{50} (Rabbit) MRID 00055602	>10 g/kg ♂	4
Acute Inhalation LC_{50} (Rat) MRID 00015307	>61.7mg/L	4
Primary Eye Irritation (Rabbit) MRID 00014686	**Non-irritant**	**4**
Primary Dermal Irritation (Rabbit) MRID 00015305	PIS = 0.1 (Intact and abraded skin)	4
Dermal Sensitization (Guinea pig) MRID 00119085	Negative	-

The following toxic signs were observed in the above studies:

Acute Oral LD_{50} in the Rat (81-1; MRID 00014675): Tremors, labored breathing, rhinorrhea, salivation and depression occurred within 12-24 hours after dosing males and females with Acephate Technical

(purity: 98%). Macroscopic examination revealed pulmonary edema and congestion in the nonsurvivers, but no gross abnormalities were observed in rats sacrificed at the termination of the study. **Recalculation of the LD_{50} slope and confidence limits for this study, which were submitted by the registrant December 12, 1979, showed that the LD_{50} and 95% confidence limits for males were 1447 (544-3846) mg/kg and for females were 1030 (150-696) mg/kg (MRID No. 00029686).** Doses tested: 400, 600, 900, 1350 and 2020 mg/kg in Sprague-Dawley rats.

Acute Dermal Toxicity in the Rabbit (81-2; MRID 00055602; duplicate MRID 00060859): Tremors and diarrhea were the only toxic signs observed in this study, in which two doses of Orthene Technical (Acephate; purity: 99-100%), 5 and 10 g/kg, were tested in male New Zealand White rabbits. Tremors occurred in both groups shortly after dosing. Diarrhea occurred the following day only in the 10 g/kg group. All affected rabbits (4/6) in the 5 g/kg group appeared normal one day after dosing. In the 10 g/kg group, all affected rabbits but one (or 5/6) appeared normal within 4 days after dosing. The test material caused no death, had no effect on body weights and no treatment-related changes were observed in several organs and tissues examined grossly. There was no difference in response to treatment between rabbits with intact (3/group) and abraded (3/group) skin. It was not stated in the submitted report when (if ever) the 6th rabbit was symptom-free.

Acute Inhalation Toxicity in the Rat (81-3; MRID 00015307; duplicate MRIDs 00022098 and 00029699): Tremors, ataxia and depression were observed in all male and female (Sprague-Dawley) rats after exposure to the test material, 61.7 mg/L (nominal concentration), but the animals appeared normal during the following day and the entire 14-day observation period. There were no deaths. The test material was an aerosolized aqueous solution of Orthene Specialty Concentrate (Orthene Technical; purity or Acephate content: 97%). The exposure time (nose only) was 4 hours. Although the concentration of the test material in the exposure chamber was not determined analytically, it seems reasonable to assume that, with a nominal concentration of 61.7 mg/L, the limit dose of 2 mg/L was reached. Although the diameters of the aerosolized particles were not determined, respirable particles were apparently present in concentrations high enough to cause toxic signs generally associated with the inhibition of cholinesterase activities in plasma, erythrocytes and brain.

Primary Eye Irritation in the Rabbit (81-4; MRID 00014686): New Zealand White rabbits with **washed eyes** had mild iritis, moderate conjunctival redness and mild to moderate chemosis at 1 hour after exposure. Slight corneal opacity in one rabbit and slight to

severe conjunctival discharge in all rabbits appeared on day 1 after exposure. Another rabbit developed a slight corneal opacity 2 days after exposure. All symptoms disappeared on the observation day 3, except for the conjunctival redness and discharge (score according to Draize scoring system was 1), which were still present in one rabbit on day 7, but not on day 14. The eyes were washed after 5 minutes of exposure.

All rabbits with **unwashed eyes** had slight to moderate conjunctival redness and discharge, and chemosis, also at 1 hour after exposure. Chemosis was seen in only one rabbit on the observation day 1, whereas conjunctival redness and discharge were present in one rabbit on day 7. All eyes were clear on day 14. There was no corneal opacity or iritis in this group. The test material was Orthene Technical (Acephate; purity: 90-98%), 100 mg/eye.

Primary Dermal Irritation in the Rabbit (81-5); MRID 00015305): At 24 hours after exposure, 2 rabbits (New Zealand White strain) had well defined erythema on both intact and abraded sites. At 48 hours, a very slight erythema was still present on the intact back of one rabbit, but it was absent at 72 hours. The test material was Orthene Specialty Concentrate (Acephate Technical content: 97%), 0.5 g, moistened with 0.5 mL of water.

Dermal Sensitization in the Guinea Pig (81-6; MRID 00119085 and 00154885): Acephate Technical (purity: 99%), diluted to 35% w/w with saline (maximal nonirritating concentration), was not a sensitizing agent to male Hartley guinea pigs under the conditions of the Modified Buehler Test. Positive results (skin irritation) were obtained with 1-chloro-2,4-dinitrobenzene (DNCB; 0.1% w/w in saline), a known sensitizer. The test involved 10 topical applications over a 22-day period on the shaved right flanks (induction phase) and, 2 weeks later, one application on the left flanks (challenge dose). Each application was made in a volume of 0.4 mL and the exposure time, under occlusion, was 6 hours.

b. Subchronic Toxicity

In this special cholinesterase (ChE) inhibition study (MRID 40504819), Sprague-Dawley rats (about 45 days old at the start of dosing), 30 males and 30 females/group, received Acephate Technical (purity: 98.2%) in the diet for 13 weeks at the nominal doses of 0, 2, 5, 10 and 150 ppm. The actual intake of the test material was 0, 0.12, 0.21, 0.58 and 8.90 mg/kg/day, respectively, for males and 0, 0.15, 0.36, 0.76 and 11.48 mg/kg/ day, respectively, for females. Cholinesterase activities in brain, erythrocytes (RBC) and plasma were determined on 10 rats/ sex

during weeks 4, 9 and 13. Other parameters examined for all rats studied were signs of toxicity, body weights (weekly) and necropsy.

Relative to the control values, Acephate Technical had no effect on body weights and no toxic signs were observed in this study. Tissue abnormalities were not observed at necropsy and there was no mortality.

Brain ChE activity was significantly ($p<0.01$) inhibited in the 2 ppm group, during week 13 in the males (7%) and during weeks 9 and 13 in the females (9% each). In the remaining groups, brain ChE activity was significantly ($p<0.01$) inhibited at all times as follows: 5-10%, 10-16% and 44-53% in the 5 ppm, 10 ppm and 150 ppm groups, respectively. The inhibitions were similar in males and females. Erythrocyte ChE activity was significantly inhibited (32-48%; $p<0.01$) only in the 150 ppm group, in males during weeks 4 and 9, and in females during weeks 9 and 13. Plasma ChE activity was significantly inhibted (43%; $p<0.01$) only in the 150 ppm females and only during week 13.

Based on the inhibitions of ChE activities, the NOELs and LOELs for male and female rats are as follows: Brain, < 2 ppm (mg/kg/day: 0.12 ♂ and 0.15 ♀) and 2 ppm (LDT), respectively; RBC, 10 ppm (mg/kg/day: 0.58 ♂ and 0.76 ♀) and 150 ppm (mg/kg/day: 8.90 ♂ and 11.48 ♀), respectively; and Plasma, 10 ppm and 150 ppm, respectively. Based on the deliberations of the Hazard Identification Committee on October 30 and December 11, 1997, the NOEL for brain ChE inhibition is 2 ppm (see HED Document No. 012453).

This study is classified as ACCEPTABLE-nonguideline (a special cholinesterase inhibition study).

In this 21-day dermal toxicity study [MRID 44541101], Acephate Technical [97.8% a.i.] was administered to 10 Sprague-Dawley rats/sex/dose via the skin [≈10% of the body surface area] at dose levels of 0, 12, 60, and 300 mg/kg/day for 21 days [6 hours per day, 5 days per week for 3 consecutive weeks]. Dose selection was based on the findings of a 5-day pilot study conducted with groups of three rats/sex/dose dermally exposed to 5, 50, 150 or 300 mg/kg/day. In this study, there were no effects on survival, clinical signs or body weight and there was no indication of dermal irritation at any dose. However, at the highest dose tested, females displayed a decrease in both plasma (83% of control) and brain (86% of control) cholinesterase while brain cholinisterase in the high-dose males was 90% of control.

In the main 21-day study, all rats survived until study

termination, and there were no clinical signs of toxicity. There was no dermal response. No adverse effects were observed on body weight, food consumption, hematology, clinical chemistry, and organ weights, and gross and microscopic findings were comparable among the groups for both sexes. At the high-dose level, both sexes displayed slight decreases in plasma and RBC cholinesterase values at study termination, although statistical significance was not attained. There was a decrease in brain cholinesterase activity in the mid- and high-dose groups of both sexes compared to the controls, and the decrease was significant (mid-and high dose females and high dose males) and dose-related. **The systemic NOAEL is 12 mg/kg/day, based on a slight decrease in brain cholinesterase activity at the systemic LOAEL of 60 mg/kg/day in females. The dermal toxicity NOAEL is 300 mg/kg/day, the highest dose tested.**

This 21-day dermal toxicity study is classified Acceptable, and it satisfies the guideline requirement for a repeated dose [21-day] dermal toxicity study [§82-2] in rats.

In this 4-week inhalation toxicity study (MRID 40504818), Acephate Technical (purity assumed to be 100%) was administered (whole body exposure) at 0 (house air only), 1.05, 10.8, and 93.6 mg/m^3 (MMAD: 1.57-2.25, 2.65-3.60 and 1.98-3.22 um, respectively; GSD: 1.79-3.28, 1.77-2.21 and 1.80-2.78, respectively) to Fischer 344 [CDF(F-344)/CrlBR] rats (25/sex/group for controls, 15/sex/ group for low-dose, 10/sex/group for mid-dose, and 20/sex/group for high-dose). The main exposure period consisted of 21 six-hour exposures over a 30-day period (10 animals/sex/group). All animals were rinsed in tepid tap water after exposure, to reduce topical exposure to Acephate. Five animals/sex from control and low-dose groups received 12 exposures over a 16-day period, at which time they were sacrificed for determination of plasma, erythrocyte, and brain cholinesterase (ChE) activities. In addition, 10 animals/sex from control and high-dose groups were retained for 4 additional weeks after cessation of exposure (recovery group).

Rats were observed twice daily for mortality and moribundity. Modified physical examinations (for toxic and pharmacologic effects) were performed after removal from exposure chamber following each exposure. Animals were weighed and detailed physical examinations were performed weekly. Food consumption was determined twice weekly for the first two weeks and weekly thereafter. Ophthalmic examinations were preformed pre-test, during week 4 for all main study animals, and during week 8 for recovery animals. Plasma and erythrocyte ChE activities were determined pre-test and at day 16 (control and low-dose rats), day 17 (all groups from main study), at termination of main study, at test day 44 and at termination of recovery phase. Brain ChE

activity was determined at day 16 (control and low-dose groups), at termination of main study, and at termination of recovery phase (control and high-dose groups). Hematological and clinical chemistry parameters were measured at termination of the main study and at termination of recovery phase (control and high dose groups). All animals received gross necropsy at study termination. All main study animals from control and high-dose groups were examined histopathologically. In addition, gross lesions, nasal turbinates, trachea, lungs and eyes from low-dose and mid-dose groups (main study), and recovery phase animals were examined histopathologically.

At post-exposure observations, high-dose males and females exhibited tremors and increased secretory responses (data not provided). In addition, 2 high-dose females exhibited tremors, and 6 exhibited polypnea during clinical observations (no other groups demonstrated these findings). On ophthalmic examination, 7/10 high-dose females exhibited miosis at week 4, and 2/10 females in the recovery phase exhibited miosis at week 8. Although no males exhibited miosis at week 4, 2/10 had this response at week 8.

Body weights were significantly less for high-dose females than control females at several time points during the study; body weights for mid-dose females were significantly decreased at week 4 only. Body weight gains were significantly decreased for high-dose males and females during weeks 0-4. There were no significant differences in food consumption among groups. There were scattered significant changes in hematological and clinical chemistry parameters.

Brain ChE activity was significantly decreased in all treated animals at all time points, except for low-dose males at days 29-30 (range: 29-36% of controls for high-dose, 62-68% of controls for mid-dose, and 62-93% for low-dose during treatment; 83-84% of controls for high dose after 4 week recovery period). Plasma and erythrocyte ChE activities were significantly inhibited for mid- and high-dose groups for males and females during the treatment phase (62-90% of controls for mid-dose plasma, 29-68% of controls for high-dose plasma; 80-85% of controls for mid-dose erythrocyte, 27-33% of controls for high dose erythrocyte). Erythrocyte ChE activity was inhibited in low-dose males and females on day 16 only (88-89% of control levels). During the recovery phase, plasma ChE activity was significantly inhibited in high-dose males only at day 44 (89% of control levels); erythrocyte ChE activity was inhibited in high-dose males and females (82-84% of control levels) at day 44; neither plasma nor erythrocyte ChE activities remained inhibited at day 59 for either sex.

There were no treatment-related gross pathological findings.

Histopathological examination demonstrated increased incidence of "induced exudate in the lumen, suppurative inflammation, individual cell necrosis, and regenerative epithelium of the middle and posterior sections of nasal turbinate (nasal passages)" of high-dose males and females. After 4-week recovery period, histopathological findings included "reduced cellularity and intraepithelial cysts of the middle and posterior sections of nasal turbinates". No similar lesions were found in the mid-dose group.

Based on the results of this study (tremors, miosis, decreased body weight and weight gain, and histopathological findings), the systemic LOEL is 93.6 mg/m^3 (0.0936 mg/L) and the systemic NOEL is 10.8 mg/m^3 (0.0108 mg/L). The LOEL for the inhibition of plasma cholinesterase (ChE) activity is 10.8 mg/m^3 (0.0108 mg/L), with a NOEL of 1.05 mg/m^3 (0.00105 mg/L). The LOEL for the inhibition of erythrocyte and brain ChE activities was 1.05 mg/m^3 (0.00105 mg/L), with a NOEL less than 1.05 mg/m^3.

This study is classified as ACCEPTABLE-guideline when combined with range-finding (MRID 40504817) and satellite (MRID 40645903) studies and satisfies guideline requirements for a subchronic inhalation study in rat (82-4).

In this 4-week inhalation toxicity study (MRID 40645903), Acephate (purity >99%) was administered (whole body exposure) at 0 (house air only), 0.187 and 0.507 mg/m^3 (MMAD: 2.84-3.59 and 2.43-3.14 um, respectively; GSD: 1.60-1.80 and 1.61-1.83, respectively) to Fischer 344 [CDF(F-344)/CrlBR] rats (10/sex/ group). The main exposure period consisted of 21 six-hour exposures over a 30-day period (10 animals/sex/group). All animals were rinsed in tepid tap water after exposure, to reduce topical exposure to Acephate. Five animals/sex from control and low dose groups received 12 exposures over a 16-day period, at which time they were sacrificed for determination of plasma, erythrocyte, and brain cholinesterase (ChE) activities. In addition, 10 animals/sex from control and high dose groups were retained for 4 additional weeks after cessation of exposure (recovery group).

Rats were observed twice daily for mortality and moribundity. Animals were weighed and detailed physical examinations were performed weekly. Food consumption was determined weekly. Ophthalmic examinations were performed pre-test and during study week 4. Cholinesterase activities in brain, plasma and erythrocytes, and hematological and clinical chemistry parameters were determined at study termination (10 animals/sex/group). All animals received gross necropsy at termination. In addition, gross lesions, nasal turbinates, and lungs were examined histopathologically.

There was a slight, dose-related, increase in urine staining of the fur in treated females when compared to controls (maximum incidence was 2 animals in each of groups 2 and 3 during week 4). In addition, two females in Group 3 demonstrated dyspnea during study week 2. The toxicological significance of these findings is questionable. There were no treatment-related changes in body weight, food consumption, clinical chemistry or hematology parameters, plasma, erythrocyte or brain ChE activities, or histopathology findings.

Based on the results of this study (lack of treatment-related effects), the systemic LOEL is >0.507 mg/m^3 (0.0005 mg/L; HDT) and the systemic NOEL is 0.507 mg/m^3. The LOEL for the inhibition cholinesterase activities in plasma, erythrocytes and brain is also >0.507 mg/m^3, with a NOEL of 0.507 mg/m^3.

This study is classified as ACCEPTABLE-guideline when combined with range-finding (MRID 40504817) and main (40504818) studies and satisfies guideline requirements for a subchronic inhalation study in rat (82-4).

c. Chronic Toxicity

In a chronic feeding study (MRIDs 00084017 [main study] and 00101623 [additional data]), Sprague-Dawley rats, 75/sex/group, received Technical RE-12420 (Acephate; purity: 92.5%) in the diet for 28 months at the following nominal doses: 0, 5, 50 and 700 ppm. Using the FDA/HEW conversion factor (1.0 ppm in food = 0.05 mg/kg/day for the older rat; Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics, 1959), these doses were equivalent to 0, 0.25, 2.5 and 35.0 mg/kg/day, respectively. No justification was presented for the selection of doses. Parameters examined for all rats in the study included daily observations, body weights, food consumption, food efficiency (during the first 8 weeks) ophthalmological examination, hematology, clinical chemistry (including cholinesterase [ChE] activities in plasma, erythrocytes [RBC] and brain), urinalysis, necropsy, histopathology of some 40 ograns/tissues (including brain, eyes, spinal cord and sciatic nerve), organ weights and organ/body weight ratios (for adrenals, brain, heart, kidneys, liver, lungs, spleen, testes/ovaries and thyroid gland - for all scheduled sacrifices). Plasma and RBC ChE activities were determined during weeks 6, 7, 19 and 28, and months 12, 18, 22, 24 and 28, using randomly selected 4, 5 or 10 rats/sex/group. Brain ChE activity was determined during weeks 7 and 19, and months 12, 22 and 28.

The following treatment-related findings were observed in the high-dose (700 ppm; 35 mg/kg/day) male rats: **(1)** Hyperactivity in some

(8%) of the males during the initial 5 months of the study; **(2)** Increased incidence of aggressive behavior (31% vs 5% in the controls), also during the initial 5 months of the study; **(3)** Decreased body weight gain (6-18%; $p \leq 0.01$) during study weeks 8-106, when compared with the controls; and **(4)** Significantly ($p \leq 0.01$) decreased food efficiency during the entire testing interval (weeks 1-8). Aggressive behavior was also observed in 13% of the low-dose (5 ppm; 0.25 mg/kg/day) and 13% of the mid-dose (50 ppm; 2.5 mg/kg/day) male rats.

Relative to the control values, plasma ChE activity was significantly ($p \leq 0.01$) inhibited at all sampling times in the high-dose males (10-50%) and females (50-72%). In the mid-dose group, the inhibitions were 0-29% for the males and 0-38% for the females. Plasma ChE activity was not inhibited in the low-dose males and slightly inhibited (0-19%) in the females. Erythrocyte ChE activity was significantly ($p \leq 0.01$) decreased at all sampling times in the high-dose males (21-67%) and females (21-61%). In the mid-dose groups, ChE inhibitions in RBC were 0-31% and 0-42% for males and females, respectively. In the low-dose group, RBC CHE activity was decreased 0-13% (males) and 0-29% (females). Relative to the control values, the inhibitions of brain ChE activity in the low-dose, mid-dose and high-dose males were 0-13%, 34-43% and 69-77%, respectively. The corresponding values for the female rats were 1-13%, 33-45% and 66-83%, respectively. Most of these inhibitions were statistically significant ($p \leq 0.01$).

Based on the neurotoxic signs, decreased body weight gain and food efficiency, and the inhibition of cholinesterase activities in plasma, erythrocytes and brain, the systemic LOEL and NOEL for the male rats are 700 ppm (35 mg/kg/day) and 50 ppm (2.5 mg/kg/ day), respectively. The systemic NOEL for the female rats is > 700 ppm. The LOEL and NOEL for the inhibition of plasma, RBC and brain ChE activities in males and females are 50 ppm (2.5 mg/kg/ day) and 5 ppm (0.25 mg/kg/day; borderline value), respectively.

This study is ACCEPTABLE-guideline and satisfies the guideline requirements for the chronic feeding study (83-1a) in the rat.

In a chronic feeding study (MRID 41812001), beagle dogs (4.0-4.5 months old), 5/sex/group, received Acephate Technical (purity: 99.9%) in the diet for one year at the following (nominal) doses: 0, 10, 120 and 800 ppm (analytical values: 0, 0.27, 3.11 and 20.16 mg/kg/day, respectively). Doses used in this study were based on the results of a 4-week preliminary study (No. HWA 2107-164) in which 8, 20, 250 500 ppm doses of Acephate Technical were tested. Parameters examined for all dogs in the current study included daily observations, physical and ophthalmological examinations,

body weight gains, food consumption and utilization, hematology, clinical chemistry (including cholinesterase [ChE] levels in plasma, erythrocytes [RBC] and brain), urinalysis, necropsy, histopathology of some 40 organs/tissues (including brain, eyes, spinal cord and sciatic nerve), and absolute and relative (organ/terminal body weight and organ/brain weight ratios) weights for 12 organs. Plasma and RBC ChE levels were determined for all dogs during the study weeks -3, -2, -1, 4, 13, 26 and 52, whereas brain ChE levels were assayed only at study termination. Substrates used for ChE determinations were acetylthiocholine (RBC and brain) and butyrylthiocholine (plasma).

The primary treatment-related effect observed in this study was the inhibition of ChE levels in brain and RBC. Relative to the control values, brain ChE levels (uMol/g) were significantly ($p<0.05$) inhibited in all male groups (17, 53 and 66%, respectively) and in the mid-dose and high-dose female groups (49 and 66%, respectively). Erythrocyte ChE levels (uMol/mL) were significantly ($p<0.05$) inhibited in the mid-dose (42-55%) and high-dose (76-87%) groups of both sexes. Plasma ChE levels (uMol/mL) were inhibited in the mid-dose (13-18%) and high-dose (6-10%) male groups and in all female groups (6-30%), but the inhibitions were dose-unrelated and statistically insignificant. Despite severe brain ChE inhibition in the mid-dose and high-dose groups of both sexes, symptoms usually associated with ChE inhibition (tremors, ataxia) were not observed.

Other treatment-related statistically significant ($p<0.05$) effects were: **(1)** Decrease in RBC count (13-26%), hemoglobin concentration (14-21%) and hematocrit (6-9%), all in the high-dose males); **(2)** Increase in activated partial thromboplastin time (34-96%), in the high-dose males; **(3)** Increase in the absolute weight of liver, in the high-dose males (29%) and females (17%); and **(4)** Perivascular infiltration and pigment in the livers (reticuloendothelial cells) of one mid-dose male and most high-dose males and females.

Based on decreases in hematological parameters (RBC, hemoglobin and hematocrit), increase in thromboplastin time, increase in absolute liver weight and histological changes in the liver (perivascular infiltration and pigment in reticuloendothelial cells), the LOEL and NOEL for systemic effects are 20.16 mg/kg/day (800 ppm; HDT) and 3.11 mg/kg/day (120 ppm), respectively (both sexes). The LOELs for cholinesterase (ChE) inhibition are as follows: Brain: 0.27 mg/kg/day (10 ppm), LDT, (males) and 3.11 mg/kg/day (females); RBC: 3.11 mg/kg/day (both sexes); and Plasma: >20.16 mg/kg/day (both sexes). The NOELs for ChE inhibition are as follows: Brain: <0.27 mg/kg/day (males) and 0.27 mg/kg/day (females); RBC: 0.27 mg/kg/day (both sexes); and Plasma: 20.16 mg/kg/day (both sexes).

This study is ACCEPTABLE-guideline and satisfies the guideline requirements for the chronic feeding study in the dog (83-1b).

d. Carcinogenicity

In a carcinogenicity study (MRID 00084017 [main study and 00101623 [additional data]), Sprague-Dawley rats, 75/sex/group, received Technical RE-12420 (Acephate; purity: 92.5%) in the diet for 28 months at the following nominal doses: 0, 5, 50 and 700 ppm. Using the FDA/HEW conversion factor (1.0 ppm in food = 0.05 mg/kg/day for the older rat; Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics, 1959), these doses were equivalent to 0, 0.25, 2.5 and 35.0 mg/kg/day, respectively. No justification was presented for the selection of doses. Parameters examined were those routinely examined in this type of a study (see Chronic Toxicity, MRID 00084017, for details). There was a higher incidence of adrenal medullary tumors (pheochromocytomas) in the treated male rats than in the control males. However, the reported incidence in the 5, 50 and 700 ppm male groups (9.7, 15.5 and 12.2%, respectively) was dose-unrelated and within the historical control range. The historical incidence of medullary tumors in the Sprague-Dawley male rats, in the testing facility, was 0.7-20.3% and the concurrent incidence was 2.7%. All of the tumors but two (one in the mid-dose and one in the high-dose group) in the current study were benign.

Based on these findings (the incidence of adrenal medullary tumors was dose-unrelated and within the historical incidence), it was conclued (by the Health Effects Division pathologist and the independent pathologist who re-evaluated all male adrenal gland histopathological sections) that Technical RE-12420 (Acephate) was not carcinogenic in this study.

This study is ACCEPTABLE-guideline and satisfies the guideline requirements for the carcinogenicity study in the rat (83-2a).

In a carcinogenicity study (MRIDs: 00105197 [main study]; and 00077209, 00105198 and 00129156 [additional data]), Charles River CD1 mice, 75/sex/group, were fed diets containing Orthene Technical (RE-12420; Acephate; purity: 92.6%) at nominal doses of 0, 50, 250 and 1000 ppm. The analytical doses were 0, 7, 36 and 146 mg/kg/day, respectively, for males and 0, 8, 42 and 167 mg/kg /day, respectively, for females. No explanation was given for the selection of dose levels. Ten mice/sex/group were sacrificed after 12 months of feeding the test material and the remaining mice, after 24 months. Parameters examined for all mice in the study included daily observations, body weight gains, food consumption,

hematology (for 10 mice/sex/group at study termination), necropsy, histopathology of some 40 organs/tissues (including brain, eyes, spinal cord and sciatic nerve) at study termination, and absolute and relative (% of body weight) weights of brain with stem, heart, liver, gonads and kidneys (at study termination). Tissues from mice which died during the study or were sacrificed moribund were also examined microscopically.

Female mice, fed 1000 ppm (167 mg/kg/day) of Orthene Technical, had higher incidence of hapatocellular carcinomas (HC) and hyperplastic nodules (HN) than did the concurrent controls. The incidence of HC in the control, 50, 250 and 1000 ppm female groups was 1.3, 1.3, 0 and 15.8%, respectively. The corresponding values for the male groups were 5.3, 2.7, 4.0 and 4.0%, respectively. All of these HC were observed at the terminal sacrifice. The incidence of HN in the control, 50, 250 and 1000 ppm groups was 2.7, 1.3, 0 and 19.7%, respectively. The corresponding values for the male groups were 13.3, 9.3, 5.3 and 17.3%, respectively. Most of the nodules (14.5 and 12.0% in the 1000 ppm females and males, respectively) were observed at the terminal sacrifice. The incidence of HC in the historical controls (22 studies; 1630 CD1 mice) ranged from 0 to 6%.

Other treatment-related findings were: **(1)** Liver lesions (hypertrophy of hepatocytes, karyomegaly and intracellular inclusion bodies) in the mid-dose (250 ppm) and high-dose (1000 ppm) males and females; **(2)** Lung lesions (dark pigmented alveolar macrophages, eosinophilic foreign bodies and alveolar hyalinosis) and lesions in nasal cavity (acute rhinitis) in the mid-dose and high-dose males and females; **(3)** Significantly ($p \leq 0.01$) decreased body weight gains in the mid-dose males (8-11%) and females (6-14%) during the study weeks 52-104, and in the high-dose males (15-30%) and females (14-29%) during the study weeks 13-104, when compared with the controls; and **(4)** Significant ($p \leq 0.01$) changes in organ weights at the high-dose level in the males (smaller livers and kidneys) and the females (larger livers and smaller kidneys, brains and ovaries), when compared with the controls.

Based on decreased body weight gains, decreased (in males) or increased (in females) weights of livers, decreased weights of kidneys, and non-neoplastic lesions in liver and lungs, the systemic LOEL is 250 ppm (mg/kg/day: 36 ♂ and 42 ♀) and the systemic NOEL is 50 ppm (mg/kg/day: 7 ♂ and 8 ♀). Based on the increased incidence of hepatocellular carcinomas in the 1000 ppm (167 mg/kg/day; HDT) females, Orthene Technical (Acephate) was carcinogenic to female mice in this study.

This study is ACCEPTABLE-guideline and satisfies the guideline

requirements for the carcinogenicity study in the mouse (83-2b).

The carcinogenic potential of Acephate was evaluated by the Health Effects Division Reference Dose (RfD)/Peer Review Committee in January, 1985 and by the Science Advisory Panel (SAP) in February, 1986. Following the EPA Proposed Carcinogenicity Assessment Guidelines (FR 11/23/84), the Committee placed Acephate in Category C (possible human carcinogen). This classification was based on the increased incidence of hepatocellular carcinomas, observed only at the terminal sacrifice and only in the high-dose (1000 ppm; 167 mg/kg /day) female mice. Although the mouse bioassay (MRID 00105197) was considered a limited evidence of carcinogenicity, Category C was used because Acephate was moderately mutagenic in the *in vitro* assays (it was not mutagenic in the *in vivo* assays). In February, 1986, SAP concluded that Acephate could be in C or D Category for carcinogenicity and that the 1000 ppm (167 mg/kg/day) dose, at which tumors (hepatocellular carcinomas) were observed, appeared to exceed the MTD (Maximally Tolorated Dose) with respect to body weights. In October, 1997, the Hazard I.D./ SARC (Science Assessment Review Committee) accepted the existing assessment of the carcinogenic potential for Acephate. The Cancer Potency Estimate (Q^*_1) is no longer required. The $Q^*_1 = 6.9x10^{-3}$, first established in December, 1984, was changed to $Q^*_1 = 9.1x10^{-3}$ in January, 1985 and was "abolished" by OPP Policy Group in October, 1985 (The Group recommended to remove Q^*_1 from the Registration Standard for Acephate, published in 1987).

e. Developmental Toxicity

In a developmental (teratology) study (MRID 41081602), virgin female rats (Crl:CD®(SD)BR strain) received, by gavage, Acephate Technical (Purity: 99.7% a.i.; Lot No.: SX-1725) in deionized water from gestation days (g.d.) 6 through 15 and were sacrificed on g.d. 20. The doses used were 0, 5, 20 or 75 mg/kg/day.

The following findings were observed in the high-dose and mid-dose groups: **(1)** Decreased body weights and body weight gains (% of control for body weight gain): 47-84 during g.d. 6-16; 80-90 [uncorrected for gravid uterine weight] and 37-71 [corrected for gravid uterine weight] during g.d. 6-20; and 86-92 [uncorrected for gravid uterine weight] and 71-84 [corrected for gravid uterine weight] during g.d. 0-20; and **(2)** Decreased food consumption and food efficiency (% of control for food consumption): 73-92 during dosing; 81-93 during g.d. 6-20; and 87-96 during g.d. 0-20. Decreases in body weights, body weight gains and food consumption were statistically significant ($p \leq 0.01$); decreases in food efficiency were not analyzed statistically. There was also a

statistically significant (≤0.01) increase in the number of rats with tremors and decreased motor activity in the high-dose group.

Developmental toxicity was noted in the high-dose group as slight decreases in the mean number of ossified caudal vertebrae, sternal centers, metacarpals, and the forelimb and hindlimb phalanges (with the hindlimb phalanges significantly reduced [p<0.05).

Based on reduced body weights, body weight gains, food consumption and food efficiency, the maternal toxicity LOEL is 20 mg/kg/day and the NOEL is 5 mg/kg/day. Based on decreases in mean numbers of ossification centers per litter, the developmental toxicity LOEL is 75 mg/kg/day and the NOEL is 20 mg /kg/day.

This study is classified as ACCEPTABLE-guideline and satisfies the guideline requirements for a developmental (teratology) study in the rat (83-3a).

In this developmental toxicity study (83-3b; MRIDs: 00069684 [main study] and 00069683 [pilot study]), artificially inseminated and then chorionic gonadotropin-injected (to induce ovulation) Dutch Belted rabbits, 16/group, received by gavage 0, 1, 3 and 10 mg/kg/day of Technical RE-12420 (Acephate; purity: 92.8%) from gestation day (g.d.) 6 through 27. The test material was administered as an aqueous solution at a constant volume of 1 mL/kg of body weight. Doses selected for this study were based on the results of the pilot study in which doses of 3, 10, 30 and 100 mg/kg/day of Technical RE-12420 (purity: 92.8%) were tested; (40% deaths and 10% weight loss were observed on g.d. 24 in the 30 mg/kg group). In the current study, the rabbits were observed daily and weighed every 6 days, and also on day 28 before they were sacrificed. The following parameters were examined at study termination: **(1)** Gross necropsy on the dams; **(2)** Determination of the uterine weights, number of implantations, postimplantation losses, resorptions, corpora lutea/dam, living and dead fetuses, and sex and body weights of fetuses; and **(3)** Examination of fetuses for malformations, variations and skeletal defects.

Two rabbits in the 10 mg/kg group aborted and were sacrificed and discarded without examination, one on gd 25 and another on gd 27. A slight increase in nasal discharge, possibly treatment-related, was observed in the 3 and 10 mg/kg groups, when compared with the controls. With the exception of these two findings, Technical RE-12420 had no effect on the maternal and developmental (teratogenic, fetotoxic) parameters examined.

Based on 2/16 (12.5%) abortions in the high-dose group and none in

the controls, the LOEL and NOEL for maternal toxicity are 10 mg/kg/day (HDT) and 3 mg/kg/day, respectively. The NOEL for developmental toxicity is > 10 mg/kg/day.

This study is ACCEPTABLE - guideline and satisfies the guideline requirement for the developmental (teratology) toxicity study in the rabbit (83-3b).

f. Reproduction

In this 3-generation reproduction study (83-4; MRIDs: 40323401 [main study] and 40605701 [corrections]), Charles River rats, 30 males and 30 females/group, were fed diets containing Acephate Technical (purity: 98.7%) for 75 days before they were bred to produce F_{1a}, F_{1b}, F_{2a} and F_{2b} litters. Because of low fertility in all groups, including the controls, for the F_{1b} and F_{2b} litters, a third generation (F_{3a}) was produced from the F_{2b} litters. All rats were continuously exposed to the test material or the control diets either directly in their feed or through the mothers' milk during lactation. The nominal doses used were 0, 25, 50 and 500 ppm, and were based on the results of an earlier (1983) rat reproduction study (MRID 00129508) in which a reproductive NOEL was not determined. Using the FDA/HEW conversion factor (1 ppm in food = 0.05 mg/kg/day, for the older rat; Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics, 1959), these doses were equivalent to 0, 1.25, 2.5 and 25 mg/kg/day, respectively. Parameters examined were those routinely examined in a multigeneration rat reproduction study.

Treatment-related effects were observed only in the 500 ppm group and included: **(1)** Decreased body weights and/or weight gains for adult males (in each generation) and females (in some generations) and for pups in the F_{2a} and F_{3a} generations; **(2)** Increases in food consumption for males and females during the premating period and decreases in food consumption for females during the gestation and lactation periods; **(3)** Clinical signs in males (increased incidence of alopecia in the first generation and increased incidence of soft or liquid stools in the second and third generations); **(4)** Decreases in mating performance for the F_{2b} generation; **(5)** Decreases in mean litter size (25-30%, $p<0.01$) for the F_{1b}, F_{2a}, F_{2b} and F_{3a} generations; and **(6)** Significant ($p<0.01$) decreases in pup survival to day 4 for the F_{1a} (3.2%) and the F_{2a} (6.3%) generations.

Based on decreased body weights and/or weight gains for adult males (each generation), and for adult females and pups (some generations), decreased food consumption during gestation and lactation periods, and decreases in litter size (some generations), the parental LOEL and NOEL are 500 ppm (25 mg/kg/

day) and 50 ppm (2.5 mg/kg/day), respectively. Based on decreases in viability index (two generations) and in mating performance (one generation), the reproductive LOEL and NOEL are also 500 ppm (25 mg/kg/day) and 50 ppm (2.5 mg/kg/day), respectively.

This study is ACCEPTABLE-guideline and satisfies the guideline requirement for a reproduction study in the rat (83-4).

g. Mutagenicity

Fourteen ACCEPTABLE-guideline studies (8 in the category of gene mutations, 2 structural chromosome aberrations and 4 concerned with other genotoxic effects) are summarized below. These studies satisfy the guideline requirement for a mutagenicity study (84-2) in the three categories listed above.

Gene Mutations

(1) Salmonella typhimurium reverse gene mutation assay (MRID 00119080): Independent tests with both the Technical (purity: 92.9%) and Analytical (purity: 99.3%) grade Acephate were positive in S. typhimurium strain TA100 in both the presence and absence of S9 activation at high levels (10000-50000 ug/plate). There was no mutagenic response in TA1537 or TA98 up to the highest dose tested (HDT) of the technical product (10000 ug/plate +/-S9).

(2) Salmonella typhimurium/Escherichia coli reverse gene mutation assay (MRID 00028625): Independent tests with Acephate Technical (purity: 93.5%) were positive with S. typhimurium strain TA100 in both the presence and absence of S9 activation at high levels (5000-10000 ug/plate). A positive response was also seen in E. coli WP2 at 5000 and 10000 ug/plate +/-S9. There was no mutagenic response in TA1535, TA1537, TA1538 or TA98 up to the HDT with these strains (5000 ug/plate +/-S9).

(3) Salmonella typhimurium reverse gene mutation assay (MRID 00132948): Six samples of Technical Acephate with varying purity (92.6 to >99%) were positive in this partial Ames test using only S. typhimurium strain TA100 in the absence of S9 activation at 50000 ug/plate. The >99% pure test substance was cytotoxic at concentrations >500 ug/plate and positive at 500 ug/plate. There was no trend of decreased genotoxicity with increased purity.

(4) Salmonella typhimurium reverse gene mutation assay (MRID 00132947): Seven of eight samples of Technical Acephate with varying purity (85-99.6%) were positive in this partial Ames test in S. typhimurium strain TA100 in the absence of S9 activation at 50000 ug/plate. The eighth sample (purity: 100%) was negative in strain TA100 and all samples were negative in strains TA1537 and TA98 up to the HDT (50000 ug/plate). S9 activation was not used in this study.

(5) Saccharomyces cerevisiae reverse mutation assay (MRID 00132949): Independent tests were positive for the induction of gene mutations in S. cerevisiae D7. Dose-related increases in the

mutation frequency (MF) were seem at test levels of 2-5% in the presence of S9 activation. The test material used in this study was Technical Acephate (purity: 93.5).

(6) Mouse lymphoma L5178Y TK+/- forward gene mutation assay (MRID 00132950): The test was positive with Technical Acephate (purity: 93.5%). Generally dose-related increases in the MF were seen at ≥2000 ug/mL - S9 or ≥3000 ug/mL + S9. Cytotoxicity occurred at the HDT (5000 ug/mL +/- S9).

(7) Mouse lymphoma L5178Y TK+/- forward gene mutation assay (MRID 00137738): The test was positive with Technical Acephate (purity: 93.5%); dose-related increases in the MF were calculated at all assayed concentrations (2429-5000 ug/mL +/- S9).

(8) In vivo mouse somatic cell mutation assay (MRID 40209101): The test was negative for the induction of somatic cell mutations in the offspring of pregnant C57B1/B6 female mice administered dietary concentrations of 200, 600 or 800 ppm of Technical Acephate (purity: 98%) during gestation days 8.5-12.5. Mortality occurred in 23% of the high-dose dams; other signs of maternal toxicity (tremors, hunched back and labored breathing) were seen in the 600 ppm and 800 ppm dams. Decreases in the percentage of pregnant females (36-37%) and percentage of pups surviving to day 28 (25-26%) were also observed at 600 and 800 ppm.

Chromosome Aberrations
Somatic Cells

(9) In vivo micronucleus assay (MRID 00132953): The test was negative in Swiss male mice receiving oral gavage doses of 75, 150 or 300 mg/kg of Technical Acephate (purity: 93.5%) once daily for two days. No deaths were observed and clinical signs, if any, were not reported. The highest dose used in this study was based on a published oral LD_{50} value for Acephate in mice (361 mg /kg).

Germinal Cells

(10) Mouse dominant lethal assay (MRID 00119081): The test was negative in male CD-1 mice receiving dietary concentrations of Technical Acephate (purity: 98.4%) of 50, 500 or 1000 ppm for 5 days (equivalent to 5.8, 60 or 71 mg/kg/day). Toxicity was manifested as decreased body weight (18% lower than control) and decreased food consumption (52% of control) in the high-dose group. A 22% reduction in the pregnancy index was also seen at the HDT, but it was statistically insignificant.

Other Genotoxic Effects

(11) DNA damage/repair in Salmonella typhimurium assay (MRID 00132955): The test was negative in DNA repair deficient strains up to the HDT (5000 ug/plate; spot test). S9 activation was not used in this study. The test material was Technical Acephate (purity: 93.5%).

(12) Saccharomyces cerevisiae recombination and gene conversion assay (MRID 00132949): Independent tests were positive in S. cerevisiae D7 at both endpoints. Dose-related increases in mitotic recombination and gene conversion were seen at Acephate concentrations of 1-5% in the presence of S9 activation. Dose-related increases in recombination and gene conversion were also seen at nonactivated levels of 3-5%. The test material was Technical Acephate (purity: 93.5%).

(13) In vitro sister chromatid exchange (SCE) in Chinese hamster ovary (CHO) cell assay (MRID 00132954): The test was positive with significant increase in the frequency of SCEs at the highest levels of Technical Acephate (purity not reported) tested (2000 ug/mL - S9; 5000 ug/mL + S9).

(14) Unscheduled DNA synthesis (UDS) in cultured WI-38 human fibroblasts assay (MRID 00028625): The test was positive at high concentrations of Technical Acephate ($\geq$1000 ug/ml) but only in the absence of S9 activation. The purity of the test material was 93.5%.

Overall, the results from the in vitro studies indicated that Technical Acephate was mutagenic in bacteria, yeast and cultured mammalian cells. Acephate aslo caused recombination and gene conversion in yeast, SCE in a cultured mammalian cell line and UDS in human fibroblasts. In general, genotoxicity was limited to high concentrations and exogenous metabolic activation (S9 microsomal fraction) was not required to uncover the positive responses. Attempts to characterize the mutagenic component(s) of Acephate by investigating a series of Acephate samples of varying purities in the Ames test failed; mutagenicity in these studies did not decrease with increasing purity levels of the test material. Nevertheless, the data from the in vivo assays with Acephate clearly showed that the genotoxic activity of Acephate was not expressed in whole animals. Confidence in the negative findings, particularly for the mouse somatic cell and the dominant lethal assays, are high because of the response induced in the target organ. The Science Assessment Review Committee (SARC) concluded, therefore, that the negative findings from the in vivo studies lessen the concern for a potential mutagenic hazard.

h. Metabolism

In this metabolism study (MRID 00014994), male and female Sprague-Dawley rats were intubated daily with nonradioactive Orthene (Acephate; analytical grade; 25 mg/kg) for 7 consecutive days. On day 8, the animals were dosed with radioactive Acephate (S-methyl-^{14}C-Orthene; purity: >99.5%; 25 mg/kg) and were sacrificed 3 days later.

Acephate was rapidly and completely absorbed from the stomach and was rapidly excreted in urine. About 87% and 95% of the administered radioactivity (^{14}C) was excreted, respectively, during the first 6 and 12 hours after dosing. Most of the remaining ^{14}C was found in the exhaled air (probably CO_2; 1-4.5%), feces (1%) and tissues (0.4%). The ^{14}C found in urine was unchanged Acephate (O,S-dimethyl acetylphosphoramidothioate; 73-77%), DMPT (O,S-dimethyl phosphorothioate; 3-6%) and S-Methyl acetylphosphoramidothioate; 3-4%). Methamidophos (O,S-dimethyl phosphoramidothioate; ORTHO 9006) was not detected in urine, and the author concluded that Methamidophos was only a plant and soil metabolite of Acephate. Of the 0.4% ^{14}C recovered in tissues, most (0.13-0.26%) was in the liver and least (0.001-0.004%) in the brain. Male and female rats had the same excretion pattern.

This study is ACCEPTABLE-nonguideline. It provides the information on the metabolism of Acephate by the rat, but does not satisfy (even partially) the guideline requirement for the metabolism studies (85-1).

The purpose of this metabolism study (MRID 00014219) was to investigate whether Methamidophos (ORTHO 9006) was formed from Orthene (Acephate) in rats. Six-week old male and female Sprague -Dawley rats were dosed (gavage) with nonradioactive Acephate (purity: 99.94%) at 100 mg/kg for 4 days. Two rats were sacrificed 3 hours after each dose (except the third) and the whole carcasses were quickly frozen and then analyzed (by GLC) for Acephate and Methamidophos. In addition, 3 male and 3 female rats were sacrificed 3 hours after the fourth dose for Acephate and Methamidophos analyses in tissues. Excreta were collected for analyses (by GLC) during the 24 hours following the third dose. The rats were sacrificed at 3 hours after being dosed because it was estimated that Methamidophos would be at or near maximum concentration at that time.

Acephate was rapidly absorbed and rapidly eliminated by the rats. The carcasses contained only 12-48% and the gastrointestinal tracts 3-14% of the final dose at 3 hours after dosing. The excreta (chiefly urine) contained 54-56% of the final dose at 6 hours after dosing. There was no tendency for Acephate to concentrate in

blood, liver, muscle, fat, heart and brain.

Rats converted a portion of Acephate to Methamidophos. Evidence was presented that the conversion took place in the small intestine and, to a lesser extent, in the stomach, and was apparently effected by the microorganisms. Methamidophos was then absorbed from the stomach and intestines, and distributed throughout the body. At 3 hours after the last dose, the carcass contained 0.6-1.6% and the excreta (chiefly urine) 1.1-1.5% of the final dose of Acephate as Methamidophos. There was no tendency for Methamidophos to accumulate in blood, liver, muscle, fat and heart. Concentrations of Methamidophos in these tissues varied from 0.2 to 1.1 ppm. Highest concentrations of Methamidophos were found in kindeys (4.1-11.5 ppm), testes (2.4-3.9 ppm) and brain (2.1-2.5 ppm).

This study is ACCEPTABLE-nonguideline. It provides information on the metabolism of Acephate by the rat, but does not satisfy (even partially) the guideline requirement for the metabolism studies (85-1). New metabolism studies, as detailed in the December 24, 1989 guidelines, are therefore required.

i. Neurotoxicity

1. Acute delayed neurotoxicity in hens

In this acute delayed neurotoxicity study (81-7; MRID 00154884), 53-week old white leghorn hens were intubated with single doses of the following test substances: water (negative control), Acephate Technical, 785 mg/kg and TOPC (tri-o-tolyl phosphate; positive control), 600 mg/kg. Acephate (purity: 99%) was administered in water and TOPC (purity:95%) in corn oil. After the initial dosing, the hens were observed for 21 days and then the negative control group and the Acephate-treated group were redosed with water and Acephate (785 mg/kg), respectively. Both groups were sacrificed 21 days later (on study day 43), whereas the TOPC-treated group was sacrificed after study day 21. All Acephate-treated hens received also an intramuscular injection of atropine sulfate at dosing and at 4, 8, 12 and 21 hours after dosing. The dose of 785 mg/kg (LD_{50}) was selected by the sponsor and was based on the results of two acute oral LD_{50} studies conducted in February and March, 1985 and included in the main report).

Toxic signs observed in the Acephate-treated group were: **(1)** Mortality (9/16 or 56% hens died, due to cholinergic effects, during days 3-7 after dosing); **(2)** Weight losses after initial dosing and redosing; **(3)** Diarrhea, lethargy, weakness in lower limbs, loss of coordination, wing droop and reduced reaction to sound and movement - each sign occurring at about 3 hours after

dosing and redosing, and persisting through day 10); **(4)** Ataxia (during the first 7 days after each dosing and decreasing in severity thereafter); and **(5)** Swelling (minimal) of axis cylinder of the sciatic nerve in one hen only.

In the TOPC-treated group, toxic signs (loss of coordination, weakness in lower limbs, ataxia and staggering gait) were observed during days 14-21 after dosing and increased in severity with time after exposure. Lesions (minimal to moderate) were observed mostly in the sciatic nerve and in all hens. These lesions included lymphocytic foci, swollen and fragmented axons, nerve fiber and myelin degeneration, and Schwann cell hyperplasia.
Based on the cholinergic and neurotoxic effects occurring shortly after dosing and disappearing within some 10 days and on the absence of lesions in the sciatic nerve (except for a slight swelling in one hen), Acephate Technical was negative for acute delayed neurotoxicity at 785 mg/kg (only dose tested). Based on the cholinergic and neurotoxic effects observed 14-21 days after dosing and increasing in severity with time, and on the prominent lesions in the sciatic nerve, in all hens, Tri-o-tolyl phosphate (TOPC; 600 mg/kg; positive control), caused acute delayed neurotoxicity.

This study is ACCEPTABLE-guideline and satisfies the guideline requirement for an acute delayed neurotoxicity study in the hen (81-7).

2. <u>Acute range-finding neurotoxicity in rats</u>

In this acute range-finding neurotoxicity study (MRID No.44203301), young adult, non-fasted Sprague-Dawley rats (Crl:CD®BR strain) received single gavage doses of ORTHENE® Tech. (acephate; purity: 99.4%; lot number: SX1725) as follows: **PART A:** 0 (deionized water; vehicle), 25, 50, 75, 150, 300, 450, 600 or 900 mg/kg (2♂ and 2♀/dose, for doses 0-450 mg/kg and 1♂ and 1♀/dose for the remaining doses; dosing date: 3/25-26/93); **PART B:** 0, 10 or 500 mg/kg (1♂ and 1♀/ group; dosing date: 4/5/93); and **PART C:** 0, 5 or 500 mg/kg (5♂ and 5♀/group; dosing date; 4/23/93). The rats were observed for 7 days and were sacrificed on day 8. Parameters examined included daily observations for toxic signs, daily detailed clinical examination, body temperatures and weights, and necropsy (performed only on animals found dead or killed moribund).

Both animals in the 900 mg/kg group, one (female) in the 600 mg/kg group and one (male) in the 500 mg/kg group died within 1-3 days after dosing. Toxic signs observed in the nonsurvivers within 15 min. to 6 hours after dosing were: **(1)** Gait alterations (rocking,

lurching or swaying, prostration and/or high carriage), tremors (whole body and/or forelimb/hindlimb), salivation, lacri- mation, constricted pupils and impaired air righting reflex; **(2)** Reduced forelimb/hindlimb grasp, hypoactivity, hypothermia, swel- ling of the face and exophthalmus; **(3)** Labored respiration and head twitch; **(4)** Staining (clear, yellow and/or tan) on the fore- limbs, urogenital area and around the mouth; and **(5)** Red ocular discharge, red material around the eyes and nose, and decreased urination and defecation. Most of these toxic signs persisted for 8 hours, some (like gait alterations) for 24 hours and labored breathing, until death. Macroscopic examination of the females revealed dark red contents in the ileum and a reddened cortico- medullary junction in each kidney (one female). The 900 mg/kg male had a distended and gas-filled duodenum and jejunum, a hemorrhagic thymus gland, and a reddened and enlarged mediastinal lymph node. No gross lesions were observed in the 500 mg/kg male which apparently died from blood loss caused by a pulled out claw.

Toxic signs observed in the surviving male and female rats were similar to those observed in the nonsurvivers. These signs occurred at dose levels of 25-900 (HDT) mg/kg. No toxic signs were noted at the two other levels of ORTHENE® Tech. tested, 5 and 10 mg/kg. The minimum effect dose levels (LOELs) and the estimated times of peak effect for each predominant sign are summarized below:

Toxic Signs Observed in Rats Sacrificed on Day 8 After Single Dosing

Toxic Sign	Min. Effect Dose (mg/kg)		Peak Effect (min.)	
	Males	Females	Males	Females
Gait alterations	25	25	90-120	90-120
Tremors	75	50	90	90
Constricted pupils	25	25	3 hrs.	4 hrs.
Lacrimation	50	25	90	90-150
Exophthalmus	50	25	90-150	90-150
Salivation	300	50	2-3 hrs.	2-3 hrs.
Hypoactivity	150	300	6 hrs.	5 hrs.
Impaired air righting reflex	150	150	90	4 hrs.
Decreased body temperature	25	25	2-4 hrs.	2-4 hrs.
Decreased body weight gain	300	150	-	-

Based on the above data, the LOEL and NOEL for neurotoxic effects,

for both sexes, are 25 mg/kg and 10 mg/kg, respectively, and the highest nonlethal dose is 500 mg/kg. It was, therefore, recommended that **(1)** the highest dose for the main acute neurotoxicity study with rats (81-8; MRID 44203303) should not exceed 500 mg/kg of ORTHENE® Tech. and **(2)** the time of peak effect should be 150 minutes after dosing. These recommendations are supported by data reported in this range-finding study.

In an additional acute range-finding neurotoxicity study (MRID 44203302), young adult, fasted (about 18 hours) Sprague-Dawley rats (Crl: CD®BR strain) received single gavage doses of ORTHENE® Tech. (acephate; purity: 99.0%; lot number: SX1725) as follows: **Phase I:** 0 (deionized water; vehicle), 5, 25, 125 or 500 mg/kg (2♂ and 2♀/group; dosing date: 9/22/95) and **Phase II:** 0, 0.5, 2.5 or 5.0 mg/kg (5♀/group; dosing date: 10/25/95). All rats were killed at 2.5 hours after dosing. Parameters examined included observation for toxic signs, body weights (on day -1, prior to dosing and prior to sacrifice), brain and brain regions weights, and cholinesterase activities (at the termination of the study) in plasma, erythrocytes (RBC), and brain regions (hippocampus, mid- brain, brain stem, cerebellum and cortex).

There were no unscheduled deaths in this study; body, brain and brain region weights were not affected at all dose levels; and no clinical signs were observed in the 0.5-5.0 mg/kg groups.

Treatment-related toxic signs in the 25 mg/kg group were tremors of the mouth (repetitive movement) and twitching of both ears. These signs were observed at the terminal sacrifice (2.5 hours after dosing) in one male rat.

The most prominent findings in the 125 mg/kg male and female groups were tremors of the mouth, forelimbs/hindlimbs and/or whole body; altered gait (rocking, lurching or swaying); and salivation and twitching of both ears. These signs were first observed at 1-2 hours after dosing and were still present at study termination.

The most prominent findings in the 500 mg/kg males and females were the same as those observed in the 125 mg/kg group, plus hypothermia and hypoactivity. These signs were also first observed at 1-2 hours after dosing.

Cholinesterase (ChE) activities, determined at the termination of the study (2.5 hours after dosing), were inhibited in a dose-related manner, in males and females, as follows: **(1)** In plasma, at dose levels of 2.5 mg/kg (F) and 5.0 mg/kg (M), and above; **(2)** in RBC, at dose level of 5.0 mg/kg and above; and **(3)** in brain, at dose level of 0.5 mg/kg and above.

Based on the clinical signs, **the NOEL and LOEL for systemic toxicity**, for both sexes, were 5 mg/kg and 25 mg/kg, respect-ively. Based on the ChE activities data, the NOELs and LOELs for ChE inhibitions were:

Plasma ChE NOEL = 0.5 mg/kg (F) and < 5.0 mg/kg (M; LDT);
LOEL = 2.5 mg/kg (F) and 5.0 mg/kg (M).

RBC ChE NOEL = 2.5 mg/kg (F) and < 5.0 mg/kg (M);
LOEL = 5 mg/kg (both sexes).

Brain ChE NOEL = 0.5 mg/kg (F) and < 5 mg/kg (M);
LOEL = 2.5 mg/kg (F) and < 5.0 mg/kg (M).

3. Acute neurotoxicity in rats

In the acute neurotoxicity study (MRID 44203303), ORTHENE® Technical (Acephate; purity: 99%) was administered in a single gavage dose to groups of 30 male and 30 female non-fasted Sprague -Dawley rats (Crl:CD® BR strain). The doses used (0, 10, 100 or 500 mg/kg) were based on the results of two range-finding studies(MRID 44203301 and 44203302) and were administered as solutions in deionized water. Parameters examined included: **(1)** Daily observations for changes in clinical condition - for all animals; **(2)** Body weights before dosing, on dosing day (day 0), and on days 7 and 14 or 15 after dosing - for all animals; **(3)** Functional observational battery (FOB), for 12 animals/sex/group - before dosing, at 2.5 hours after dosing ("peak effect"), and on study days 7 and 14; **(4)** Locomotor activity (MA), after the completion of the FOB; **(5)** Cholinesterase (ChE) activities in plasma, erythrocytes (RBC) and 6 brain regions (brain stem, cerebellum, cortex, hippocampus, midbrain and olfactory), for 6 animals/sex/group/sampling time - before dosing, at 2.5 hours after dosing, and on study days 7 and 14; **(6)** Whole and regional brain weights for all ChE animals; **(7)** Whole brain weights and brain dimensions for the FOB/MA animals; and **(8)** Microscopic examination of selected central and periph- eral nervous tissues from 5 animals/sex in the control and 500 mg /kg FOB/MA group, at the termination of the study (day 15).

The following treatment-related findings were observed in the 500 mg/kg and 100 mg/kg male and female groups: **(1)** Whole body and/or limb tremors; ataxia, weakness in hindlimbs and repetitive movement of mouth and jaws; alterations in posture, gait and mobility; low arousal and no approach and touch responses; decreased rearing and motor activities, rotarod performance, and body temperature; increased righting reflex and time to first step;

and lacrimation, salivation and soiled fur; **(2)** Decreased body weight gains in males only (41-45% and 15% in the high-dose and mid-dose groups, respectively); and **(3)** Inhibition of cholin- erase activities in plasma (86-88%), RBC (53-55%) and brain (the six regions tested: 83-88%). Findings observed only in the 500 mg/kg male and female groups were: Increased catalepsy time and clonic convulsions; absence of the pinch, startle, pupil and olfactory responses; decreased hindlimb footsplay and forelimb and hindlimb grip strength; chromodacryorrhea; and clear or colored (tan, red, brown and/or yellow) staining/matting material on various body surfaces.

The following treatment-related findings were observed in the 10 mg/kg male and female groups: Whole body tremors (single occur- rences) in one male and one female; inhibition of ChE activities in plasma (31-34%), RBC (18-19%) and brain regions (37-48%); and decreased rotarod performance in males on day 0 (when compared with that of the controls).

Toxic signs occurred within 0.5-2.5 hours after dosing and persisted for 4-8 hours or longer, but were not observed during the next day (study day 1). Plasma and RBC ChE activities were inhibited significantly ($p<0.01$) only during the dosing day. Brain ChE activities were inhibited ($p<0.01$) during dosing day (all regions), day 7 after dosing (all regions but olfactory) and day 14 (midbrain only). Other parameters examined in this study were not affected by ORTHENE® Tech.

Based on the above findings, the LOEL and NOEL for neurotoxicity, for both sexes, are 10 mg/kg (LDT) and <10 mg/kg , respectively. The LOELs and NOELs for the inhibition of plasma, RBC and brain cholinesterase activities are also 10 mg/kg and <10 mg/kg, respectively.

This study is ACCEPTABLE - guideline and satisfies the guideline requirement for an acute neurotoxicity study in the rat (81-8).

4. Subchronic neurotoxicity in rats

In this subchronic neurotoxicity study (MRID 44203304), Acephate (purity: 99%) was administered to Sprague Dawley rats (30/sex/group) at 0, 5, 50, or 700 ppm in the diet (mean compound intake was 0.33, 3.31, and 48.63 mg/kg/day for males, 0.41, 3.95, and 58.27 mg/kg/day for females, respectively) for 13 weeks. Body weights were recorded weekly, food consumption was recorded twice weekly, and clinical observations were recorded daily. Cholinesterase activity was determined in plasma, erythrocytes, and brain (6 regions) at weeks 3, 7, and at study

termination in 6 animals/sex/group. Neurobehavioral assessment (functional observation battery and motor activity testing) was performed in 12 animals/sex/group prior to compound administration and during study weeks 3, 7, and 12. Brain weights (whole brain and regional) were determined during study weeks 3, 7, and at study termination in non-perfused animals (6/sex/group). At study termination, 12 animals/sex/group were euthanized and perfused in situ for neuropathological examination; brain weights and measures were determined. Of the perfused animals, 5/sex for control and 700 ppm groups were subjected to histopathological evaluation of brain and peripheral nervous system tissues.

The only effects seen at the 5 ppm dose were inhibition of brain cholinesterase (significant in at least one sex for all brain regions, inhibition ranged from 2 to 28%).

At 50 ppm dose, there was significant inhibition of brain cholinesterase in all regions for both sexes (ranging from 18-55%). Plasma cholinesterase was inhibited at 50 ppm for males and females at week 3 (25-41%). Erythrocyte cholinesterase was not significantly inhibited, but was decreased by 26% in females at week 3. Thus, the NOEL for plasma cholinesterase inhibition was 5 ppm, with a LOEL of 50 ppm. Other effects seen at 50 ppm included a slight increase in clinical signs, specifically hair loss.

At the 700 ppm dose, brain and plasma cholinesterase were significantly inhibited in both sexes at all time points (range 55-74% inhibition for plasma, 63-82% inhibition for brain). Erythrocyte cholinesterase was significantly inhibited in both sexes at all time points (37-46%) except for week 13 females (25% inhibition). Thus, the NOEL for erythrocyte cholinesterase was 50 ppm, with a LOEL of 700 ppm. Additional effects seen at 700 ppm included decreased body weight (males) and body weight gain (males and females); increased food consumption (when measured as g/kg/day); increased grooming, increased rearing, and decreased rotarod time in males; decreased motor activity in females.

Based on the effects seen in this study, the LOEL for systemic effects (increases in clinical signs) was 50 ppm (3.31 or 3.95 mg/kg/day for males or females, respectively), with a NOEL of 5 ppm (0.33 or 0.41 mg/kg/day for males and females, respectively). The LOEL for neurotoxicity (FOB findings and decreased motor activity) was 700 ppm (48.63 or 58.27 mg/kg/day for males or females, respectively), with a NOEL of 50 ppm (3.31 or 3.95 mg/kg/day for males or females, respectively). The LOEL for erythrocyte cholinesterase inhibition was 700 ppm (48.63 or 58.27 mg/kg/day for males or females, respectively), with a NOEL of 50 ppm (3.31 or 3.95 mg/kg/day for males or females, respectively). The LOEL for

plasma cholinesterase inhibition was 50 ppm (3.31 or 3.95 mg/kg/day for males or females, respectively), with a NOEL of 5 ppm (0.33 or 0.41 mg/kg/day for males and females, respectively). The LOEL for brain cholinesterase inhibition was 5 ppm (0.33 or 0.41 mg/kg/day for males and females, respectively), with the NOEL less than 5 ppm (the lowest dose tested).

The study is classified as ACCEPTABLE-guideline and satisfies the guideline requirement for a subchronic neurotoxicity study in the rat (82-7).

j. Other Toxicological Considerations

Dermal Absorption Study (MRID 00154886): In this study, male Sprague-Dawley rats (age: 144-151 days; weight: 498-618 g), 4/dose/exposure period, received single applications of a mixture of Acephate Technical and radioactive (^{14}C) Acephate, and were sacrificed after 0 (immediately after dosing), 2, 8 and 24 hours of exposure. The purity of Acephate Technical was 98.7% and the radiochemical purity of ^{14}C-Acephate was 99.1%. The concentrations of Acephate applied in 0.05 mL of a dosing solution (distilled H_2O + 0.1% w/w Tween 80) were 0.5 mg (4421000 dpm) and 5.0 mg (4435000 dpm) per rat or 0.899 mg/kg and 9.333 mg/kg (actual mean values), respectively. Acephate was labeled in the carbon atom of the $*CH_3S$- group of the molecule. After the applications on the intact (shaved) dorsal trank, the rats were housed singly in metabolic cages and had unlimited access to food and water.

Acephate Technical was absorbed slowly through the intact skin of the male rats. At 24 hours after dosing, the recovery of applied radioactivity (expressed as ^{14}C-Acephate) was 78.3% and 90.6% in the 0.5 mg/rat and 5.0 mg/rat groups, respectively. Most of this radioactivity was recovered from the surface of the skin (application site). Systemic absorption was defined as the percentage of the recovered dose in the carcass, blood, urine, feces, CO_2 trap and cage wash. In the 0.5 mg/rat group, 2.1, 3.0 and 10.5% of the recovered dose (radioactivity) was absorbed in 2, 8 and 24 hours, respectively. The corresponding values for the 5.0 mg/rat group were 1.6, 3.6 and 7.6%, respectively. Most of the absorbed radioactivity was found in urine (6.0% in the low-dose group and 4.4% in the high-dose group at 24 hours after exposure). Systemic absorption was not examined immediately after dosing (0 time).

This study is ACCEPTABLE-guideline and satisfies the guideline requirement for a dermal absorption study in the rat (85-2).

Domestic Animal Safety Study (86-1): This study is not

required because, based on the use pattern of Acephate, significant exposure for domestic animals is unlikely.

k. Reference Dose

Since February, 1989 until October, 1997, the Reference Dose (RfD) for Acephate was 0.004 mg/kg/day. This value was based on the LOEL of 2 ppm (0.12 mg/kg/day; LDT) for the inhibition of brain ChE activity in the 90-day feeding study with rats (special ChE inhibition study; MRID 40504819) and the uncertainty factor (UF) of 30. On October 30, 1997, the Health Effects Division (HED) Hazard Identification Committee reassessed the existing RfD for Acephate and recommended that the SF of 100 (rather than 30) be used with the LOEL of 0.12 mg/kg/day (which the Committee regarded as a NOEL because the inhibitions of ChE activities at different time intervals were small [1-9%], dose-unrelated and not always statistically significant). With the SF of 100, the newly established RfD for Acephate is 0.0012 mg/kg/ day.

l. Toxicology Endpoints and Dose Levels for Use in Risk Assessments

Based on comprehensive evaluation of the toxicology data available for Acephate, the following toxicology endpoints and dose levels were identified by the HED Hazard Identification Committee for use in risk assessments:

Acute Dietary Exposure: Critical study: Acute neurotoxicity range-finding study with rats (MRID 44203302; Nonguideline). NOEL: 0.5 mg/kg, based on the inhibition of ChE activities in plasma and brain of female rats. Recommended UF: 100.

Chronic Dietary Exposure: Critical study: 90-day rat feeding study (special ChE inhibition study; MRID 40504819; Nonguideline). NOEL: 2 ppm (0.12 mg/kg/day),based on the inhibition of brain ChE activity. Recommended UF: 100.

Short-Term, Intermediate-Term and Long-Term Dermal Exposures: Critical study: 21-Day dermal toxicity study with Technical Acephate in rats; (MRID 44541101; Guideline No. 82-2). NOEL: 12 mg/kg/day, based on the inhibition of brain ChE activity. Recommended UF: 100.

Inhalation Exposure: Critical study: 4-week inhalation toxicity study in rats (MRID 40645903; Guideline No. 82-4). NOEL: >0.507 mg/cubic meter (0.0005 mg/L). Recommended UF: 100.

Aggregate Risk: Because of similarity of the endpoints,the

Committee recommended that separate margin of exposure be calculated for dermal and inhalation exposure.

m. Food Quality Protection Act (FQPA) Considerations

Persuant to the language and intent of the FQPA directives regarding infants and children, the applicable toxicity data base for Acephate was evaluated by the HED Hazard Identification Committee and the Committee concluded the following:

Adequacy of data: There are no data gaps for the assessment of the effects of Acephate following *in utero* and/or early postnatal exposure. Suitable studies are **(1)** Three-generation reproduction study in rats (MRID 40323401); **(2)** Developmental toxicity study in rats (MRID 41081602); **(3)** Developmental toxicity study in rabbits (MRID 00069684); and **(4)** Mouse somatic cell assay (MRID 40209101).

Susceptibility issues: There was no indication of an increased sensitivity of the offspring of rats, mice and rabbits to prenatal and postnatal exposure to Acephate. In all studies examined, maternal or parental NOELs were smaller than or similar to offspring NOELs.

Uncertainty factor: The Committee determined that, for Acephate, the 10-fold uncertainty factor for the protection of infants and children would be removed. This decision was based on the following considerations:

(1) There are no data gaps for the assessment of the effects of Acephate on young animals. Also, a developmental neurotoxicity study with rats was not required for Acephate.

(2) The available data demonstrated no indication of an increased sensitivity of the offspring (rats, mice and rabbits) to Acephate following the prenatal and/or postnatal exposure.

n. Toxicity Data Gaps

The only data gap currently is the metabolism study with rats (85-1). The existing studies (MRIDs 00014994 and 00014219) provide information on the metabolism of Acephate by the rat, but are 25 years old and do not satisfy (even partially) the guideline requirements for the metabolism studies (85-1).

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